Copy for the Elected Office (EO/US) PCT/AU95/00875

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

FISHER ADAMS KELLY
Santos House
Level 15
215 Adelaide Street
G.P.O. Box 1413
Brisbane, QLD 4000
AUSTRALIE

Date of mailing (day/month/year)
29 August 1996 (29.08.96)

Applicant's or agent's file reference
2/3306/PC

IMPORTANT NOTIFICATION

International application No.
PCT/AU95/00875

International filing date (day/month/year)
22 December 1995 (22.12.95)

1. The following indications appeared on record concerning:

[ ] the applicant [ ] the inventor [X] the agent [ ] the common representative

Name and Address

GRANT ADAMS & COMPANY
Santos House
Level 15
215 Adelaide Street
G.P.O. Box 1413
Brisbane, QLD 4000
AUSTRALIA

State of Nationality | State of Residence

Telephone No.

Facsimile No.

Teleprinter No.

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [X] the name [ ] the address [ ] the nationality [ ] the residence

Name and Address

FISHER ADAMS KELLY
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215 Adelaide Street
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AUSTRALIA

State of Nationality | State of Residence

Telephone No.

Facsimile No.

Teleprinter No.

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Ting Zhao

Telephone No.: (41-22) 730.91.11

Form PCT/IB/306 (March 1994) 001207430

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

CULLEN & CO.
G.P.O. Box 1074
Brisbane, QLD 4001
AUSTRALIE

Date of mailing (day/month/year)
30 October 1996 (30.10.96)

Applicant's or agent's file reference
961533IDJMP

IMPORTANT NOTIFICATION

International application No.
PCT/AU95/00875

International filing date (day/month/year)
22 December 1995 (22.12.95)

1. The following indications appeared on record concerning:

[ ] the applicant [ ] the inventor [X] the agent [ ] the common representative

Name and Address
FISHER ADAMS KELLY
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2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

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3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer
Ting Zhao

Telephone No.: (41-22) 730.91.11

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and Trademark Office
(Box PCT)
Crystal Plaza 2
Washington, DC 20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 28 August 1996 (28.08.96)	
International application No. PCT/AU95/00875	**Applicant's or agent's file reference** 2/3306/PC
International filing date (day/month/year) 22 December 1995 (22.12.95)	**Priority date** (day/month/year) 22 December 1994 (22.12.94)
Applicant CHAM, Karim, Rouan	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

22 July 1996 (22.07.96)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Ting Zhao
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 730.91.11

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 961533IDJMP	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International application No. **PCT/AU 95/00875**	International filing date 22 December 1995	Priority Date 22 December 1994
International Patent Classification (IPC) or national classification and IPC **Int. Cl.**[6] A61M 1/36		
Applicant ARUBA INTERNATIONAL PTY LTD (et al.)		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of sheet(s).

3. This report contains indications relating to the following items:

I	☒	Basis of the report
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☒	Lack of unity of invention
V	☒	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☐	Certain observations on the international application

Date of submission of the demand 22 July 1996	Date of completion of the report 18 December 1996
Name and mailing address of the IPEA/AU AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No. (06) 285 3929	Authorized Officer **R CHAO** Telephone No. (06) 283 2191

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/AU 95/00875

I. Basis of the report

1. This report has been drawn on the basis of *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.)*:

[X] the international application as originally filed.

[ ] the description, pages , as originally filed,
pages , filed with the demand,
pages , filed with the letter of ,
pages , filed with the letter of .

[ ] the claims, Nos. , as originally filed,
Nos. , as amended under Article 19,
Nos. , filed with the demand,
Nos. , filed with the letter of ,
Nos. , filed with the letter of .

[ ] the drawings, sheets/fig , as originally filed,
sheets/fig , filed with the demand,
sheets/fig , filed with the letter of ,
sheets/fig , filed with the letter of .

2. The amendments have resulted in the cancellation of:

[ ] the description, pages

[ ] the claims, Nos.

[ ] the drawings, sheets/fig

3. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

4. Additional observations, if necessary:

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

- [ ] restricted the claims.
- [ ] paid additional fees.
- [ ] paid additional fees under protest.
- [ ] neither restricted nor paid additional fees.

2. [ ] This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

- [X] complied with.
- [ ] not complied with for the following reasons:

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

- [X] all parts.
- [ ] the parts relating to claims Nos.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	3, 5-14, 19-22	YES
	Claims	1, 2, 4, 15-18	NO
Inventive step (IS)	Claims	3, 5-14, 19-22	YES
	Claims	1, 2, 4, 15-18	NO
Industrial applicability (IA)	Claims	1-22	YES
	Claims		NO

2. Citations and explanations

Citations

(a) WO 95/03840 A1 (THE UNIVERSITY OF QUEENSLAND) 2 February 1995

(b) US 4895558 (CHAM) 23 January 1990

NOVELTY (N): Claims 1, 2, 4, 15-18

Citation (a) explicitly discloses all of the features of claims 1, 2, 4 and 15-18 as defined. See in particular page 6 lines 8 to 34 to page 11 line 3. The citation clearly describes a method whereby the solvent extraction step is carried out separately and remotely from the subject thereby overcoming some of the listed prior art deficiencies. It is further noted that although present claim 1 defines a method capable of the removal of cholesterol ... as a discontinuous flow system the citation at page 3 line 37 and page 7 lines 20-24 discloses of a "semi-continuous" process which is considered to infer the system is discontinuous as defined.

Similarly page 6 of the citation details known solvent extraction procedures whereby liquid-solvent extraction systems have been conducted manually (lines 16-34) this is also considered to define a "discontinuous system", with the solvent extraction step clearly being carried out remote from the subject.

Citation (b) clearly discloses a method for the removal of lipids from animal plasma, the method comprises the steps of drawing blood from the subject, separating the plasma and red blood cells dilapidating the plasma with a lipid solvent, remixing the dilapidated blood plasma with the red blood cells, and re-introducing the dilapidated blood to the subject (see column 3). The method similarly carries out the solvent extraction step separately and remotely from the subject as defined in claim 1.

The cited document is considered to be capable of operating as a discontinuous flow system by switching on/off the centrifugal separator or blood pumps or by having to provide refill bags of Prime solution or Anticoagulant solution during treatment.

It is noted the present specification describes major disadvantages of cintinuous systems as being the explosive nature of the solvents and their proximity to the subject and medical staff, and the unreliability of continuous systems in washing out the solvent because continuous systems cannot provide a sequential multi-washes (pages 5 and 6). The cited

Supplemental Box

(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of : Box V

documents both disclose of the solvent extraction occurring remote from the subject. Neither of the cited documents specifically describes a multi-wash system per se, however citation (b) at column 8 lines 18-32 discloses of the plasma/solvent mixture passing through a separator where most of the solvent-lipid combination is removed from the plasma, next Ether is added to the plasma to remove any remaining solvent from the plasma after the separation unit. This is considered to provide a "multi-wash" discontinuous solvent separation equivalent. It is also noted that the method steps of present claim 1 do not define any such "multi-wash' procedures allegedly associated only with discontinuous flow systems as described in the specification.

INVENTIVE STEP (IS): Claims 1, 2, 4, 15-18

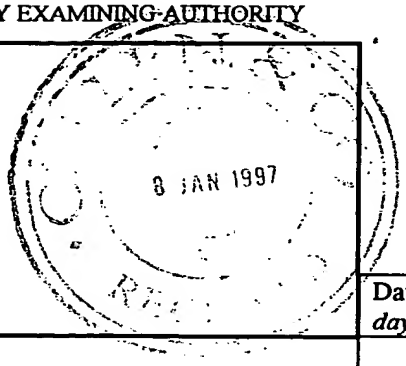
As above.

PATENT COOPERATION TREATY

From the:
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

Cullen & Co.
GPO Box 1074
BRISBANE QLD 4001



PCT

NOTIFICATION OF TRANSMITTAL OF INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing *day/month/year*	7 JAN 1997
Applicant's or agent's file reference 961533IDJMP	**IMPORTANT NOTIFICATION**

International application No.	International filing date	Priority date
PCT/AU 95/00875	22 December 1995	22 December 1994

Applicant

ARUBA INTERNATIONAL PTY LTD (et al.)

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translations to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide

Name and mailing address of the IPEA/AU	Authorized officer
AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No.: (06) 285 3929	**R CHAO** Telephone No. (06) 283 2191

Form PCT/IPEA/416 (July 1992)copbko

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61M 1/36	A1	(11) International Publication Number: WO 96/19250
		(43) International Publication Date: 27 June 1996 (27.06.96)

(21) International Application Number: PCT/AU95/00875

(22) International Filing Date: 22 December 1995 (22.12.95)

(30) Priority Data:
PN 0307 22 December 1994 (22.12.94) AU

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(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: CHAM, Karim, Rouan [NL/AU]; 353 Woodlands Drive, Sheldon, QLD 4157 (AU).

(74) Agent: GRANT ADAMS & COMPANY; Santos House, Level 15, 215 Adelaide Street, G.P.O. Box 1413, Brisbane, QLD 4000 (AU).

(81) Designated States: AL, AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, UG, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, LS, MW, SD, SZ, UG).

Published
With international search report.

(54) Title: TREATMENT FOR CARDIOVASCULAR AND RELATED DISEASES

(57) Abstract

A method for the removal of cholesterol, triglycerides and other lipids from animal plasma, serum or other suitable blood fractions, as a discontinuous flow system, the method comprising withdrawing blood from a subject, separating the required fraction from the blood and mixing with a solvent mixture which extracts the lipids form the fraction, after which the delipidated fraction is recombined with the blood cells and returned to the subject, characterised in that the solvent extraction step is carried out separately and remote form the subject. The delipidated fraction is washed with a second solvent before being recombined with the blood cells. To ensure that the delipidated fraction is free from all extraction solvent, the fraction is mixed with an absorbent specific for the solvent that is being removed. The preferred absorbent is a macroporous polymeric bead contained in the pores of a sintered glass or plastic sphere, the bead being capable of absorbing organic molecules from an aqueous solution. By treating the plasma, serum or other suitable blood fraction of a patient by these methods, the blood rheology of a patient with impaired blood circulation can be improved. Further, a rapid regression of coronary atherosclerosis occurs.

TITLE

TREATMENT FOR CARDIOVASCULAR AND RELATED DISEASES

TECHNICAL FIELD

THIS INVENTION relates to plasma or serum delipidation in animals (which term shall indicate humans), to a treatment for cardiovascular disease and to removal of excess fat from the animals. In particular, it is directed to the removal of cholesterol, triglycerides and other lipids, and fat soluble toxins - for example, insecticides - from the blood plasma or serum of such animals.

BACKGROUND ART

Cardiovascular diseases are responsible for a significant number of deaths in most industrialised countries.

One such disease is atherosclerosis which is characterised by local fatty thickening in the inner aspects of large vessels supplying blood to the heart, brain and other vital organs. These lesions obstruct the lumen of the vessel and result in ischaemia of the tissue supplied by the vessel. Prolonged or sudden ischaemia may result in a clinical heart attack or stroke from which the patient may or may not recover.

The relationship between dietary lipid, serum cholesterol and atherosclerosis has long been recognised. In many epidemiological studies it has been shown that a single measurement of serum cholesterol has proved to be a significant predictor of the occurrence of coronary heart disease.

Thus diet is the basic element of all therapy for hyperlipidaemia (excessive amount of fat in plasma). However, the use of diet as a primary mode of therapy requires a major effort on the part of physicians, nutritionists, dietitians and other health professionals.

If dietary modification is unsuccessful, drug therapy is an alternative. Several drugs, used singly or in combination, are available. However, there is no direct evidence that any cholesterol-lowering drug can be safely administered over an extended period.

A combination of both drug and diet may be required to reduce the concentration of plasma lipids. Hypolipidaemic drugs are therefore used as a supplement to dietary control.

Many drugs are effective in reducing blood lipids, but none work in all types of hyperlipidaemia and they all have undesirable side effects. There is no conclusive evidence that hypolipidaemic drugs can cause regression of atherosclerosis. Thus, despite progress in achieving the lowering of plasma cholesterol to prevent heart disease by diet, drug therapies, surgical revascularization procedures and angioplasty, atherosclerosis remains the major cause of death in Western Countries.

In view of the above, new approaches have been sought to reduce the amount of lipid in the plasma of homozygotes and that of heterozygotes for whom oral drugs are not effective.

Plasmapheresis (plasma exchange) therapy has been developed and involves replacement of the patient's plasma with donor plasma or more usually a plasma

protein fraction. This treatment can result in complications due to the possible introduction of foreign proteins and transmission of infectious diseases. Further, plasma exchange removes all the plasma proteins as well as very low density lipoprotein (VLDL), low density lipoprotein (LDL), and high density lipoprotein (HDL).

It is known that HDL is inversely correlated with the severity of coronary arterial lesions as well as with the likelihood that these will progress. Therefore, removal of HDL is not advantageous.

Known aphaeresis techniques also exist which can remove LDL from plasma. These techniques include absorption of LDL in heparinagarose beads (affinity chromatography) or the use of immobilised LDL-antibodies. Other methods presently available for the removal of LDL involve cascade filtration absorption to immobilised dextran sulphate and LDL precipitation at low pH in the presence of heparin. Each method specifically removes LDL but not HDL.

LDL aphaeresis has, however, disadvantages. Significant amounts of other plasma proteins are removed during aphaeresis and to obtain a sustained reduction in LDL-cholesterol, LDL aphaeresis must be performed frequently (up to once weekly). Furthermore, LDL removal may be counter productive as low blood LDL levels may result in increased cellular cholesterol synthesis.

To satisfy the need for a method of achieving a reduction in plasma cholesterol in homozygous familial hypercholesterolemia, heterozygous familial hypercholesterolemia and patients with acquired hyperlipidaemia other than by diet, drug therapy, LDL

aphaeresis, or a combination of these, an extra corporeal lipid elimination process, termed "cholesterol aphaeresis", has been developed. In cholesterol aphaeresis, blood is withdrawn from a subject, plasma separated from the blood and mixed with a solvent mixture which extracts lipid from the plasma, after which the delipidated plasma is recombined with the blood cells and returned to the subject.

In more detail, cholesterol aphaeresis results in the removal of fats from plasma or serum. However, unlike LDL aphaeresis, the proteins that transport the fat (apolipoproteins) remain soluble in the treated plasma or serum. Thus the apolipoproteins of VLDL, LDL and HDL are present in the treated plasma or serum. These apolipoproteins, in particular apolipoproteins A1 from the defatted HDL in the plasma or serum, are responsible for the mobilisation of excessive amounts of deposited fats such as cholesterol in arteries, plaques, or excessive amounts of triglycerides, adipose tissue, or fat soluble toxins that are present in adipose tissue. These excessive amount of fats or toxins are transferred to the plasma or serum, bound to the newly assembled lipoproteins. Thus by applying another cholesterol aphaeresis procedure, these unwanted fats or toxins are successively removed from the plasma and thus the body.

The main advantage of this procedure is that LDL and HDL are thus not removed from the plasma but only cholesterol, some phospholipids and considerable triglycerides. United States Patent No 4,895,558 describes such a system.

While cholesterol aphaeresis has overcome the shortcomings of dietary and/or drug treatments and other aphaeretic techniques, existing apparatus for cholesterol aphaeresis does not provide a sufficiently rapid and safe process. For use in a clinical setting, apparatus is required which effects delipidation more efficiently. Furthermore, flow rates of the order of 70 ml/min are required for cholesterol aphaeresis of a human subject.

Thus the cholesterol aphaeresis described in the afore-mentioned US Patent No 4,895,558 was improved by incorporating into the system a spinner to disperse the incoming plasma laterally into the extracting solvent in the form of fine droplets to improve separation efficiency. This improved system is described in International Patent Application No PCT/AU94/00415.

Unfortunately, practice has established that the cholesterol aphaeresis systems described above still suffer from a number of disadvantages.

The first disadvantage is the explosive nature of the solvents used to delipidate this plasma. These solvents are, by the very nature of the continuous systems, in close proximity to the patient and medical staff. This hazard is clearly present for the duration of the delipidation process which usually runs for several hours.

The second disadvantage is that, in the prior continuous systems, a reliable procedure is not available to remove totally all of the solvents used in the delipidation before the treated plasma is returned to the patient.

In particular, the use of the preferred solvent 1-butanol in the delipidation is of concern as it can now be established that that solvent can be present as 1% to 5% of the treated plasma that is returned to the patient. This is because continuous systems can only include a single wash to remove solvents such as 1-butanol and a single wash is now found to be insufficient. It is not possible to provide sequential multi-washes in a continuous system because the patient would have to supply an unacceptable volume of blood to maintain each stage of the system overall and the patient would also be subjected to an increased hazard factor from the prolonged exposure to the solvents.

The long term toxicity of 1-butanol is not known, especially when directly present in the blood stream - it may cross the blood brain barrier. Certainly, external contact with this solvent is known to cause irritation of mucous membranes, contact dermatitis, headaches, dizziness and drowsiness.

A third disadvantage is that the continuous systems described above are not suitable for the delipidation of serum. If serum can be delipidated, there would be the advantage of favourably altering the blood rheology in that the viscosity will decrease following delipidation resulting in better haemodynamics for the originally impaired blood circulation.

Yet a fourth disadvantage is that delipidation in a continuous system is undertaken over several hours. Apart from the prolonged exposure to the hazardous solvents as discussed above, the equipment and staff are committed to a single patient. As the removal of

plasma or other blood fractions and their subsequent return to the patient as individual steps each only take a few minutes, it would be advantageous if the relatively lengthy delipidation step could be undertaken off site, thus freeing the patient, medical staff and equipment for other matters.

Finally, in a continuous system, clearly it is only the patient's own blood fraction that can be returned to that patient. However, for example, if the patient's plasma or serum could be removed and treated remote from the patient, then either autologous or non-autologous plasma or serum could be returned to the patient at a later date.

SUMMARY OF THE INVENTION

It is an object of the present invention to overcome, or at least ameliorate, the above-mentioned disadvantages in the provision of a method for delipidating not only plasma but also serum and other blood fractions which substantially reduces the exposure of the patient to the potentially hazardous solvents used, which also can effectively remove all traces of solvent(s) used in that delipidation, and which significantly reduces the contact time between the patient and the actual delipidation process.

It is a further object to provide a method whereby advantageous changes to the blood rheology of the originally impaired blood circulation of the patient can be achieved.

It is yet another object to provide a method whereby a patient's plasma or serum can be treated remote from that patient, thus allowing either autologous or

non-autologous plasma or serum to be returned to the patient at a later date.

In one aspect of the present invention, there is provided a method for the removal of cholesterol, triglycerides and other lipids from animal plasma, serum or other suitable blood fractions, as a discontinuous flow system, said method comprising withdrawing blood from a subject, separating the required fraction from the blood and mixing with a solvent mixture which extracts the said lipids from the fraction, after which the delipidated fraction is recombined with the blood cells and returned to the subject, characterised in that the solvent extraction step is carried out separately and remote from the subject.

Preferably, as part of the solvent extraction step, beads are used when mixing the blood fractions with the solvent. More preferably, the beads have a density substantially mid-way between the density of the fraction and the density of the solvent mixture. This ensures efficient mixing with a large surface area, increasing the efficiency of the extraction and also serving as a good separator of the plasma from the solvent when centrifugation is used to isolate the phases after extraction.

Preferably, to obtain a density substantially mid-way between the density of the fraction and the density of the solvent mixture, the beads contain entrapped air.

More preferably, as the density of plasma is approximately 1.006 g/ml and the solvents used generally have a density of approximately 0.8 g/ml, the density of the beads will be around 0.9 g/ml.

The beads may be manufactured from any acceptable material such as glass or plastic.

Once the resultant delipidated fraction-containing phase has been isolated, all traces of the extraction solvent must be removed before the fraction is recombined with the blood cells and/or returned to the subject.

One way of removing this solvent is to wash with another solvent, preferably diethyl ether, to remove substantially all of the original solvent used in the extraction step.

More preferably, four (4) washes are undertaken.

However, as another aspect of the present invention, efficient removal of the extraction solvent can be achieved by mixing the delipidated fraction with an absorbent specific for the solvent that is being removed.

In particular, the absorbent is contained in the pores of sintered spheres.

More preferably, the sintered spheres are approximately 2 to 5 mm in diameter with the pores of the spheres being less than 50 Å in diameter. Most preferably, the spheres are manufactured from glass.

Preferably, the absorbents used in the sintered spheres are the macroporous polymeric beads for absorbing organic molecules from aqueous solutions marketed by Bio-Rad Laboratories under the trade name Bio-Beads SM.

If the solvent used to delipidate the fraction is 1-butanol, then the absorbent is preferably Bio-Beads SM-2.

Preferably, the absorbent is held in a chamber which is adapted to allow the delipidated fraction to pass through or over the absorbent at least twice if a single pass is insufficient to remove all of the solvent.

Preferably, as part of isolating the delipidated fraction-containing phase, that phase is subsequently washed with another solvent, preferably diethyl ether, to remove a substantial amount of the original solvent before the treatment with the absorbent.

More preferably, that phase is washed at least three (3) times.

The plasma may be human plasma or plasma from other living animals. The plasma can be obtained from human or animal blood by known plasma separating techniques which include centrifugal separation, filtration and the like.

Similarly, the serum or other lipid-containing fraction can be derived from human or other living animals by known techniques.

Suitable solvents for the extraction comprise mixtures of hydrocarbons, ethers and alcohols. Preferred solvents are mixtures of lower alcohols with lower ethers. The lower alcohols suitably include those which are not appreciably miscible with the plasma and these can include the butanols (butan-1-ol and butan-2-ol). C_{1-4} ethers are also preferred and these can include the propyl ethers (di-isopropyl

ether and propyl ether). Other solvents which may be applicable include amines, esters, hydrocarbons and mixtures providing that the solvent can (1) rapidly and preferably remove cholesterol from the plasma, (2) is substantially immiscible with the plasma, (3) can be removed from the plasma, and (4) does not denature the desired moieties. Preferred solvent compositions are butanol with di-isopropyl ether and these may be in the ratio of 0% - 40% of the alcohol to 100% - 60% of the ether.

DETAILED DESCRIPTION OF EMBODIMENTS

Materials and Methods

Animals

The roosters used in this study were of White Leghorn Hiline strain and were obtained as one-day old chicks. All roosters from 8 weeks old were transferred into individual cages. Water and feed were supplied unrestricted. At eight weeks of age, 15 control birds were fed a commercial poultry ration for 31 days and another group of 30 birds were injected subcutaneously each day with 5mg diethylstilboestrol (DES) in sesame oil for a period of 31 days. In addition they were fed on the same commercial diet which was supplemented with 2.6% (w/w) cholesterol for a period of 31 days. Fifteen animals of the DES treated group were then subjected to lipid aphaeresis (LA). Fifteen animals of the DES treated group had sham treatments. Once the LA or sham treatments commenced, all animals were fed the standard poultry ration, except during the actual treatment itself when animals were kept off their feed for three hours following reinfusion of their

autologous blood. Animals were sacrificed two days following the 4th treatment, LA or sham.

Lipid Aphaeresis Procedure

Approximately 25% of the calculated blood volume was collected from a brachial vein of the animal with a 21 gauge needle and syringe. The total blood volume was estimated at 8 percent of the body weight. The blood was collected in heparinized tubes and immediately centrifuged at 900 g for 5 minutes at room temperature. The blood cells were suspended in an amount of saline equivalent to the plasma volume and were reinfused into the animal. The plasma was kept refrigerated for twelve hours and was then delipidated for 20 minutes with a mixture of butanol and di-isopropyl ether (DIPE), 25:75 (v/v), in a ratio of one volume of plasma to two volumes of butanol-DIPE mixture (organic phase). Inert plastic beads with a density of 0.9g/mL (1g) were added to the mixture. After extraction, the mixture was centrifuged at 900 g for 2 min to separate the plasma and organic phases. The organic phase (upper layer) was removed, free of plasma phase, by careful aspiration with a pasteur pipette under vacuum. Traces of butanol in the plasma phase were washed out with four volumes of diethyl ether (DEE) for 2 min by end-over-end rotation at 30 rpm. The mixture was then centrifuged at 900 g for 2 min to separate plasma and ether phases. The ether phase was subsequently removed by aspiration with a pasteur pipette. Residual ether was removed by evacuation with a water pump aspirator at 37°C. The plasma was then passed through a 5 mL column containing Bio-Beads SM-2.

This procedure yielded delipidated plasma. The delipidated plasma was re-mixed with the blood cells of a subsequent 25% blood collection which was then reinfused through a brachial vein back into the identical donor animals. The duration of the entire procedure, that is, removal of blood from the animal to reinfusion of treated blood back to the animal was approximately 1 hour. After the fourth lipid aphaeresis treatment, the animals were sacrificed and their livers and aortae were dissected. The LA treatment procedures were repeated 3 times after the first treatment.

Sham Treatment Procedures

This was essentially the same as the LA procedure with the exception of the plasma delipidation with the organic solvents. The blood was collected in heparinized tubes and immediately centrifuged at 900 g for 5 min. The plasma was separated from the blood cells. The blood cells were mixed with saline in the same volume of the collected plasma and reinfused into the animal. The plasma was kept refrigerated for twelve hours and was then remixed with blood cells of a subsequent 25% blood collection after the second and/or subsequent plasma separations. After the fourth lipid aphaeresis treatment, the animals were sacrificed and their livers and aortae were dissected. The sham treatment procedures were repeated 3 times after the first treatment.

Tissue Lipid Preparation

The livers were weighed, minced with a scalpel blade and homogenised in 0.9% sodium chloride solution by 10-12 strokes of a motor driven Teflon-glass

homogeniser (1900 rpm). The aorta was weighed and three times its weight of 3 mm glass beads were added in a homogenising bottle containing 0.9% sodium chloride. The contents were then homogenised for one minute. The lipid from the homogenised liver and aorta samples were extracted by the Folch procedure and weighed.

Table 1 Effect of LA and sham treatments on the total lipid concentrations in livers and aortae of hyperlipidaemic roosters.

	UNTREATED CONTROLS	TREATED FOUR APHAERESIS APPLICATIONS	
	n = 15	SHAM n = 15	LA n = 15
LIVER[a]	3.65 ± 0.98	5.53 ± 1.50 [b]	3.72 ± 1.00 [b]
AORTA[a]	6.01 ± 0.97	8.11 ± 2.15 [c]	6.12 ± 0.95 [c]

[a] Total lipid concentrations expressed as g lipid per 100 g tissue, mean ± SD

[b, c] p values were < 0.05 when sham treatments were compared with LA treatments.

There were no statistical differences between the values of corresponding tissues in the untreated control group and the LA treated group.

All animals were sacrificed two days after the final aphaeresis treatment.

Humans

Patients have the plasmapheresis procedure undertaken using known transvenous techniques and plasmapheresis systems.

Plasmapheresis is performed using vein-to-vein or arteriovenous fistula in the forearm of patients. Heparin is given at the beginning of the procedure as a 5,000 unit bolus, and then by continuous infusion at the rate of 700 units per hour over the course of the procedure. Access through the antecubital veins should provide plasma flow rates of 25 to 40 mls per minute.

Blood taken from a patient is immediately treated with ACD-A (anticoagulant) in a ratio of between 1:8 and 1:16 (ACD-A:blood). The plasma is separated from this solution using a conventional plasmapheresis machine.

Twenty five percent plasma is removed from the patient. This represents one percent of the ideal body weight.

Only the first volume of plasma collection is replaced with plasma replacement fluid to the patient.

The plasma is kept refrigerated up until twelve hours prior to reinfusion of delipidated plasma in exchange for another twenty five percent plasma collection (weekly or biweekly).

The plasma is delipidated and the delipidated plasma is tested to ensure all solvent has been removed

before the clean delipidated plasma is exchanged for new untreated plasma.

In one embodiment of the present invention, the continuous flow system described in US Patent No 4,895,558 (the entire content of which is included herein) is modified to a discontinuous system by removing the appropriate blood volume to be treated and subjecting that volume to delipidation at a site remote from the patient.

In another embodiment of the present invention, the continuous flow system described in International Patent Application No PCT/AU94/00415 (the entire content of which is included herein) is modified to a discontinuous system by removing the appropriate blood volume to be a site remote from the patient before the plasma is dispersed into small droplets into the solvent by the dispersing means.

In either of the above embodiments, the extraction step can include, in accordance with the present invention, either multiple washing of the extracted phase and/or using an absorbent.

For example, the plasma is delipidated with a solvent mixture comprising 1-butanol and di-isopropyl ether. The delipidated fraction is then washed three (3) or four (4) times with diethyl ether. After the final wash, the diethyl ether is removed by centrifugation and vacuum extraction at 37°C. The sintered spheres containing Bio-Beads SM-2 are then mixed with the delipidated plasma to remove the final traces of 1-butanol.

Conclusions

DES administration to the roosters resulted in a significant amount of fat (lipid) accumulation in the livers and aortae.

Discontinuous LA treatments corresponding to approximately one plasma volume treated by four applications of 25% of plasma volume treated per time resulted in significant decreases in both hepatic and aortic lipids in hyperlipidaemic animals. Moreover, the LA treated hyperlipidaemic animals ended up with lipid values that were similar to control animals.

(i) These experiments show that excessive amounts of body fats in the form of adipose tissue (triglycerides) in the liver can be removed by LA; and

(ii) regression of atherosclerosis occurs in the aorta by LA treatments.

Similar results can be expected for human patients.

By adapting the prior art methods to discontinuous flow systems, the present invention can remove or at least significantly reduce any danger to patients and medical staff from the explosive nature of the solvents employed.

Further, by using the improved solvent extraction methods of the present invention, all of the potentially poisonous extraction solvents can be removed before the treated blood is returned to the patient.

Also, the improved solvent extraction method of the present invention is not limited to plasma delipidation but also it is applicable to the delipidation of serum, thus providing advantageous changes to the blood rheology of the originally impaired blood circulation of the patient.

The present invention thus provides for a rapid regression of coronary atherosclerosis in a patient.

Finally, as the present invention is a discontinuous system, it is not essential to return the delipidated blood fraction immediately to the patient. It is already known that plasma or serum can be collected and stored under sterile conditions in a refrigerator or freezer for extended periods and that it can be returned safely to the patient within twelve (12) hours of breaking the sterile seal. Therefore, if necessary, reintroduction of the delipidated fraction can occur several weeks after it was first removed from the patient. This option leads to particular advantages such as, economies of scale when several patients have to be treated simultaneously, the freeing of medical staff and equipment for other duties, and the reduction in stress for the patient whom no longer has to be hooked up to a delipidation apparatus for several continuous hours. Further, it enables a bank of plasma or serum to be maintained which is free of any infection which can be delipidated and exchanged for a patient's plasma or serum as required. Of course, autologous or non-autologous plasma or serum could be returned to the patient under these conditions.

The embodiments are described by way of illustrative examples only and various changes and modifications

may be made thereto without departing from the inventive concept as defined in the following claims.

CLAIMS:

1. A method for the removal of cholesterol, triglycerides and other lipids from animal plasma, serum or other suitable blood fractions, as a discontinuous flow system, said method comprising withdrawing blood from a subject, separating the required fraction from the blood and mixing with a solvent mixture which extracts the said lipids from the fraction, after which the delipidated fraction is recombined with the blood cells and returned to the subject, characterised in that the solvent extraction step is carried out separately and remote from the subject.

2. A method as defined in Claim 1, wherein the extraction solvent is substantially removed from the delipidated fraction by washing with a second solvent.

3. A method as defined in Claim 2, wherein the delipidated fraction is washed four times.

4. A method as defined in Claim 2 or Claim 3, wherein the second solvent is diethyl ether.

5. A method as defined in any one of Claims 1 to 4, wherein the solvent extraction step comprises:

(a) mixing the solvent mixture containing the fraction with beads, said beads being of a density substantially mid-way between the density of the fraction and the density of the solvent mixture; and

(b) isolating the thus delipidated fraction-containing phase.

6. A method as defined in Claim 5, wherein the beads contain entrapped air to obtain the density substantially midway between the density of the fraction and the density of the solvent mixture.

7. A method as defined in Claim 6, wherein the density of the beads is about 0.9 g/ml.

8. A method as defined in Claim 1, wherein the extraction solvent is removed from the delipidated fraction by mixing the delipidated fraction with an absorbent specific for the extraction solvent.

9. A method as defined in Claim 8, wherein the absorbent is contained in the pores of sintered spheres.

10. A method as defined in Claim 9, wherein the sintered spheres are about 2mm to 5mm in diameter and the pores of the spheres are less than Å in diameter.

11. A method as defined in any one of Claims 8 to 10, wherein the absorbent is a macroporous polymeric bead for absorbing organic molecules from an aqueous solution.

12. A method as defined in any one of Claims 8 to 11, wherein the absorbent is held in a chamber which is adapted to allow the delipidated fraction to pass through or over the absorbent at least twice.

13. A porous sintered sphere for use in a method as defined in any one of Claims 8 to 12, said sphere containing an absorbent in its pores.

14. A sintered sphere as defined in Claim 13, wherein the absorbent is a macroporous polymeric bead for absorbing organic molecules from an aqueous solution.

15. A method of changing the blood rheology of an animal with impaired blood circulation whereby the plasma, serum or other suitable blood fraction of the animal has been treated by a method as defined in any one of Claims 1 to 12.

16. A method for rapid regression of coronary atherosclerosis in an animal whereby the plasma, serum or other suitable blood fraction from the animal is treated by a method as defined in any one of Claims 1 to 12.

17. A method of removing excessive adipose tissue from an animal whereby the plasma, serum or other suitable blood fraction from the animal is treated by a method as defined in any one of Claims 1 to 12.

18. A method of removing fat soluble toxins from an animal whereby the plasma, serum or other suitable blood fraction from the animal is treated by a method as defined in any one of Claims 1 to 12.

19. A method of changing the blood rheology of an animal whereby the plasma or serum of the animal is exchanged for non-autologous plasma or serum wherein said non-autologous plasma or serum has

been treated by a method as defined in any one of Claims 1 to 12.

20. A method of rapidly regressing coronary atherosclerosis in an animal whereby the plasma or serum of the animal is exchanged for non-autologous plasma or serum wherein said non-autologous plasma or serum has been treated by a method as defined in any one of Claims 1 to 12.

21. A method of removing excessive adipose tissue from an animal whereby the plasma or serum of the animal is exchanged for non-autologous plasma or serum wherein said non-autologous plasma or serum has been treated by a method as defined in any one of Claims 1 to 12.

22. A method of removing fat soluble toxins from an animal whereby the plasma or serum of the animal is exchanged for non-autologous plasma or serum wherein said non-autologous plasma or serum has been treated by a method as defined in any one of Claims 1 to 12.

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 95/00875

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: A61M 1/36

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61M 1/- B01D 11/-

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
IPC : AU as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
DERWENT : (triglycerid: or cholesterol or lipid# or extract: or solvent: or salvat:)
JAPIO : as above

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 95/03840, A1, (THE UNIVERSITY OF QUEENSLAND) 9 February 1995 See page 6 lines 8 to 34, page 8 line 36 to page 11 ine 3 and Figs. 1,2	1,2,4,15-18
X	US 4 895 558, A, (CHAM) 23 January 1990, See Abstarct,Col 3 line 27 - Col 4 line 8 and Col 8 lines 3-65, and Figure 6	1,2,4
A	DE 3310 263, A1, (FRESENIUS AG) 27 September 1984 See Entire document	1-22

☐ Further documents are listed in the continuation of Box C

☒ See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 March 1996	20TH MARCH 1996.
Name and mailing address of the ISA/AU AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No.: (06) 285 3929	Authorized officer LARS KOCH Telephone No.: (06) 283 2551

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member	
WO	9503840	AU	72605/94
DE	103310263	US	4895558

END OF ANNEX

WO 96/19250
PCT/AU95/00875

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

To:

GRANT ADAMS & COMPANY
Santos House
Level 15
215 Adelaide Street
G.P.O. Box 1413
Brisbane, QLD 4000
AUSTRALIE

Date of mailing (day/month/year) 27 June 1996 (27.06.96)	
Applicant's or agent's file reference 1895/KMP	**IMPORTANT NOTICE**

International application No.	**International filing date**	**Priority date**
PCT/AU95/00875	22 December 1995 (22.12.95)	22 December 1994 (22.12.94)

Applicant

ARUBA INTERNATIONAL PTY. LTD. et al

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:

   AT,AU,BR,CA,CN,CZ,DE,EP,FI,GB,JP,KP,KR,LK,NO,NZ,PL,RO,RU,SK,US

2. In accordance with Rule 47.1(c), third sentence, each designated Office will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Offices.

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on

   27 June 1996 (27.06.96) under No. WO 96/19250

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, a demand for international preliminary examination must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the national phase, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

9 JUL 1996

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer: J. Zahra
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 730.91.11

Form PCT/IB/308 (July 1992)

1130398

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING SUBMISSION OF PRIORITY DOCUMENTS

(PCT Administrative Instructions, Section 411)

To:

GRANT ADAMS & COMPANY
Santos House
Level 15
G.P.O. Box 1413
215 Adelaide Street
Brisbane, QLD 4000
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Date of mailing (day/month/year) 24 January 1996 (24.01.96)	
Applicant's or agent's file reference 1895/KMP	IMPORTANT NOTIFICATION

International application No.	International filing date (day/month/year)	Priority date (day/month/year)
PCT/AU95/00875	22 December 1995 (22.12.95)	22 December 1994 (22.12.94)

Applicant
ARUBA INTERNATIONAL PTY LTD et al

The applicant is hereby notified of the date of receipt by the International Bureau of the priority document(s) relating to the following application(s):

Priority application No:	Priority date:	Priority country:	Date of receipt of priority document:
PN 0307	22 Dec 1994 (22.12.94)	AU	23 Jan 1996 (23.01.96)

The International Bureau of WIPO
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